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Full Length Research Article

POSTHARVEST FUNGAL DETERIORATION OF TOMATO (*LYCOPERSICUM ESCULENTUM* MILL*)* AND PEPPER (*CAPSICUM ANNUM L*): THE "ESA" CONNECTION

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ABSTRACT
In Southwestern Nigeria, many poor, illiterate urban dwellers and commercial food vendors often intentionally use physically damaged tomatoes and pepper ("esa" in the local parlance) for their cookings. This research set out to identify fungi associated with physically damaged tomato and pepper and verify their effects on the nutritional composition of these vegetables. Healthy looking and physically damaged tomatoes and pepper were sourced from Mile 12 Market in Lagos state. Fungi (*Aspergillus flavus and Aspergillus niger*) isolated from samples of "esa" were inoculated into the healthy looking vegetables. Using standard analytical techniques, vitamins, minerals and proximate composition were determined in both the visually healthy and "esa" vegetables (at 3 and 5 days after inoculation). Mean values from 3 replicates ± S.D was reported for each parameter. Each parameter in the Control had a significantly higher (P=0.05) mean value than those of the inoculated samples. Day 3 samples in turn had a significantly higher (P=0.05) mean value for each parameter compared to the day 5 samples, with the exception of calcium and potassium. Findings from this work have debunked the myth that "esa" tomato and pepper are as good as the visually healthy ones in terms of nutritional worth. On account of the possible health implications (associated with the ingestion of mycotoxins that are usually associated with fungal species), there is the need to enact and or enforce appropriate legislations to discourage the myriad road side restaurants in Nigeria from using these physically damaged condiments in their cooking.

Keywords: E*sa*, tomatoes, pepper, proximate and vitamin composition, mycotoxins and Mile 12 Market, Lagos

INTRODUCTION

Tomatoes (*Lycopersicum esculentum)* and 'tatase' (*Capsicum annum L*) are important commodities for the preparation of many local dishes in Nigeria. They play a major role in providing vitamins and minerals for humans (Smith, 1994) and also serve as raw materials for many agro based as well as agro allied Industries. The production of the bulk of the fresh tomato and 'tatase' in Nigeria is in the Northern part of Nigeria, whereas the consumption and utilization is done all over the country. They are used either fresh or processed into paste, puree, ketchup etc.

Large quantities of tomato and 'tatase' are produced all year round in Nigeria and production tonnage is highest between August and November (Obikwe and Obaseki-ebor, 1987).

Tomato and 'tatase' are eaten in both the green and ripe state as a source of vitamins A, B, C, lycopene and other nutrients (Lucius, 1961; Rosales *et. al*, 2006).

Tomatoes are planted by an estimated 85% of the gardens each year (Olaniyi *et al*., 2010). If well managed, they are highly productive (Denton and Swarp, 1983). Cropping of tomatoes and 'tatase' during the wet and dry seasons contribute immensely to the national requirement; however, the bulk of production is from the dry season cropping (Anon, 1989).

Unfortunately, these important staple vegetables are highly perishable and deteriorate few days after harvest, losing almost all their required qualities and in some cases, resulting into a total waste of these vegetables (Okhuoya, 1996).

The losses most often are caused by microbial infection, physiological breakdown due to natural ripening processes and environmental conditions such as heat and drought (Idah *et.al.,* 2007) Furthermore, improper postharvest sanitation, poor storage and packing practices and mechanical damage during harvesting, handling and transportation (resulting from vibration by undulation and irregularities on the road during transportation) can enhance wastages (Idah *et al.,* 2007).

Over the years, different microbes (bacteria and especially fungi) have been reported as causing spoilage and deterioration of nutrients in different fruits (Willey *et. al*., 2008). When tomatoes and 'tatase' spoil as a result of the life processes of bacterial, yeasts, and molds, the sugars are rapidly used up (Willey *et.al*., 2008).

In Southwestern Nigeria, especially in the urban centres, it is a common knowledge that the resource poor urban dwellers and many commercial food vendors often intentionally purchase the physically damaged tomatoes and 'tatase' (popularly referred to in the local parlance as "esa") for their cooking because these types are much cheaper than the undamaged ones. It is also believed by these people that the damaged physical conditions of these vegetables is only of cosmetic significance, and as such, do not negatively impact on their nutritional value.

Aims and Objectives

In view of the foregoing, the aim of this research is to determine the effect of some fungal species on the nutritional worth of tomatoes (*Lycopersicon esculentum)* and 'tatase' (*Capsicum annum L*), particularly the "esa" grade. The following are the specific objectives of this work:

(1) To isolate and identify fungal species associated with physically damaged tomatoes and pepper at Mile 12 market, Lagos-State, Nigeria.

(2) To determine the effect of these fungal species on the nutritional value of infected tomatoes and pepper.

MATERIALS AND METHODS

2.1 Sampling Area

Samples of healthy and physically damaged (infected) tomatoes and 'tatase' fruits used in this study were sourced from Mile 12 market in Lagos state. This market is probably the largest and the most popular market (in terms of the sales of foodstuff) in the entire South Western Nigeria. Both kinds of sample i.e. physically healthy and physically damaged, were each collected in a separate sterile polythene bag and transported to the laboratory (within 1 hour after collection) for the isolation of fungi. Prior to isolation however, these samples were surface sterilized by dipping completely in 0.1% mercuric chloride solution for less than 1minute and immediately rinsed in 3 changes of sterile distilled water.

2.2 Isolation and Identification of Fungal Pathogens

Using a sterile forcep, 2 slices were cut from each of the samples and incubated in separate plates containing freshly prepared Potato Dextrose Agar (PDA). The plates were kept in an incubator pre set at 30^{o}C for 48 hours (or more, depending on growth of the individual plate). To obtain pure cultures of the fungal isolates, developing fungal cultures were aseptically sub cultured repeatedly into freshly prepared PDA plates until cultures consisting of only one type of fungus was obtained.

To identify each of the fungus, a small portion of fungal growth from each pure plate was teased with a sterile inoculating loop into 1-2 drops of Lacto phenol in-cotton blue on a clean slide. A cover slip was placed on it and it, and this was examined under a light microscope.

Fungal identification was done by comparing the morphological features of each of the prepared fungal slide as examined under the microscope as well as their corresponding pure plate with the descriptions given by Talbot (1971) and Deacon (1980).

2.3 Infection and Pathogenicity Test

The spore of the now already identified fungal Isolates - *Aspergillus niger* and *A.flavus* from the pure plates of the cultured physically damaged samples were each washed into sterile plates using 12 ml of sterile distilled water per pure plate. Each was immediately inoculated (under sterile condition) into fresh looking, visually healthy samples of tomato and 'tatase' that had been previously surface sterilized as described above, using a sterile 5 ml syringe and hypodermic needle. Each type of fungal isolate was introduced into each of the different samples using a separate (new) sterile syringe and needle.

At about 32 hours (after inoculating these visually healthy samples), the tomato and 'tatase' samples showed the symptoms of deterioration that were similar to those observed from the initial samples. This process of re-infection of the visually fresh and healthy samples was repeated again by isolating and culturing of the fungal pathogens from the newly infected samples and the symptoms that were observed in this instance were equally similar to what was observed previously.

The Control samples composed of visually healthy tomato and 'tatase' samples that were surface sterilized as described above, but without any fungal samples inoculated into them.

All samples were stored at room temperature (27^{o}C – 29^{o}C) and tests on nutritional composition were conducted on all samples at 3 days and 5days after inoculation. The following nutritional elements were tested for in the samples: vitamins (A, B1 and C), minerals (Ca, Mg, K, Zn and Fe) and proximate composition (moisture, ash, fat, protein, fiber, carbohydrate and energy).

2.4. Proximate Composition, Vitamins and Mineral in Samples

Proximate composition was determined according to official methods of the Association of Official Analytical Chemists (A.O.A.C., 1984). This includes determination of moisture content, ash content, crude protein, crude fiber, fat and total carbohydrate contents. Using the same methods, vitamins A, B1, C and essential minerals were also evaluated in the samples.

2.4.1 Determination of Moisture Content in Samples

The method of AOAC (1984) as modified by Onyenke (1995) that was used in determining the moisture content is the Air Oven Method (AOM).

Analytical balance was used to obtain the weight of three oven dried moisture dishes with lids. Thereafter, 2.0g of each sample (for triplicate determinations) were weighed into each of the dishes. The samples in the dishes were dried without the lids in an oven maintained at 100^{o}C for 17hours. The samples were removed after drying and the lids were replaced. The samples inside the dishes were cooled in desiccators [containing a suitable moisture absorbing material (desiccant)] at a room temperature (28^{o}C) and then weighed. The process of drying, cooling and weighing was continued until a constant weight was obtained.

2.4.2 Determination of Ash Content in Samples

A clean dry porcelain dish was weighed using analytical balance. Exactly 2g of each sample was weighed into the dish. This was dried in an oven at 100^{0}C for 3hours. The dish was heated over a Bunsen flame to initiate the destruction of carbon (the content was heated gently until it turns black). Thereafter, the dish and content was placed into a muffle furnace. The content was heated at 560^{o}C for 7hours until greyish-time residue is obtained to indicate the destruction of organic farinaceous portion of the sample. The hot dish was removed from the furnace with the use of tongs and the ashes obtained moisten with some drops of water and any unashed carbons that are still present were exposed. The remaining ash was dried again in an oven for 3hours at 560^{o}C, and thereafter in a muffle furnace for 45 minutes. The content and dish were removed from the furnace and allowed to cool, after which it was placed in a desiccator to cool completely. The dish and contents were weighed to determine the content of ash (Onyeike *et al*., 1995).

2.4.3 Determination of Crude Protein in Samples

Crude protein in both samples was determined using Kjeldahl method where 1.0g of each sample was weighed into nitrogen – free filter paper. The paper was dropped into the kjeldahl digestion flask, a few bumping chips, and 30g of digestion catalyst and 250ml of the pure concentrated nitrogen –free H_2SO_4 was added. The flask was placed in an electro thermal heater and held

in a tilting position clamped on a retort stand. The flask was heated gently thermostatically until frothing has ceased and the contents have become completely liquefied. The digestion temperature was increased and the flask was rotated occasionally to pick-up undissolved particles on the wall of the digestion flask. The heat was intensified until a clear light green colour appears. The digestion was allowed to cool at room temperature and diluted with distilled water to 100ml mark.

2.4.4 Determination of Crude Fat in Samples

As modified by FAO (1986), exactly 2.5g of each of the samples was weighed on a Whatman No542 hardened ash-less filter paper and placed into an extraction thimble. The thimble and content were placed into a 50ml beaker and dried in a mechanical convection oven at 100^0C for 7hours to expel all traces of moisture. The thimble and content were removed from the oven into the Soxhlet apparatus. The beaker was rinsed twice with the extracting solvent, which in turn was added to the Soxhlet apparatus. The sample was then extracted for 7hours at a condensation rate of 240 drops min^{-1}.After the extraction, the extract was transferred into an already weighed evaporating dish. The dish was then rinsed 3times with the extractant and the rinsing was added to the dish. This dish was thereafter placed in the fume chamber and the fan was switched on to enable the solvent to evaporate. The dish and contents were dried in an oven under temperature of 100^0C for 25minutes, then removed from the oven into a desiccator to cool and the weight of the dish and content was obtained.

2.4.5 Determination of Crude Fiber in Samples

Exactly 2.0g of each of the samples was weighed into $1dm^3$ conical flask and the sample was extracted by stirring with petroleum ether. The samples were allowed to settle so as to facilitate the decanting of the ether. This process was repeated 3 times after which it was allowed to dry in free circulating air. Approximately 200ml of 1.25% H_2SO4 was heated to boil; 50ml of it was added to the dried sample and with the dispenser, the remaining 150ml of the boiled H_2SO_4 was added. Thereafter, 6 drops of 2% silicon in CCl_4 solvent was added and heated to boil within 2minutes. The entire set up was then boiled gently for another 30minutes under the cold –finger condenser. This flask was allowed to rotate so as to mix the contents, after which 12.5cm filter paper was used to filter the contents using a Buchner funnel. Automatic dispenser was used to measure 200ml of 1.25% NaOH which has been heated to boiling point. This alkaline solution was used to wash the sample back into the initial flask and then boiled for another 30minutes under the cold-finger condenser rotating the flask to mix the contents. All the insoluble matter were transferred to the sintered crucible using boiling water. It was then washed 3 times with alcohol and then three times with dimethyl ether before drying (in an oven at 105^oC) to a constant weight. The sample was then ash dried by incineration in a muffle furnace at 560^oC for about one hour. The crucible containing the ash was then allowed to cool in a desiccator and weighed again.

2.4.6 Carbohydrate Determination

Exactly 45ml of the sample extract was diluted with 45ml of distilled water. 1ml of the diluted filtrate was pipetted into each of three test tubes (for triplicate determinations) and the same thing was done to 1ml of glucose (standard). 5ml of freshly prepared 0.10% Anthrone reagent was pipetted into each of the test tubes. The test tubes were stopped using a stopper. The tubes and the content were mixed thoroughly by gentle shaking. Each test tube was labeled and placed in a test tube rack. The test tubes and rack were placed into a water-bath (37^oC) for between 11-13minutes. The test tubes were removed from the bath and allowed to cool at room temperature (28^oC). The absorbance of the samples and standards were read in a spectrophotometer at 630nm against the reagent blanks. The total available carbohydrate was then calculated (AOAC, 1984).

2.4.7 Determination of Vitamin A in Samples:

The Antimony Trichloride Method was used to determine vitamin A according to the method of Rosenheim and Drummond, Carr and Price as reported in AOAC (1984). One gramme of the sample was weighed accurately into a volumetric flask of 10ml capacity. The sample was dissolved in chloroform and made to volume of solvent. About 0.2ml. of this solution was transferred with the aid of pipette into glass cell of 1cm internal thickness, and run in exactly 2ml. of a 30% solution of antimony trichloride in chloroform from a burette. During the addition of the reagent, the solution was gently shaken, until the blue colour produced is matching with the Rosenheim Schuster colorimeter. The final match is made at the point of maximum intensity of the blue colour. The blue colour was then correlated to blue value expressed in blue units based on the assumption that the value of pure vitamin A is equal to 80,000.

2.4.8 Determination of Vitamin B1 in Samples:

The method used in determining the vitamin B1 was the Formaldehyde-Azo Test according to Kennersley and Peter as reported by AOAC (2000). Exactly 0.5ml of the diazotized sulfanilic acid was added to 1.25ml of the sodium bicarbonate-sodium hydroxide reagent in a small test tube. After 1minute, 0.3ml of 40% formaldehyde was added. Then 0.1 - 0.3ml of the solution of vitamin B1 of acidity greater than pH 4.0 was also added. A pink colour develops slowly, and increases in intensity for 30-60 minutes, after which time it is practically constant. It was then compared in the colorimeter against the standard vitamin treated the same way.

2.4.9 Determination of Vitamin C in Samples:

Standardized Dye Method was used to determine the vitamin C content. 2, 6-Dichlorobenzeneindophenol reagent was used according to Birch, Harris, and Ray as reported in AOAC (1984). Exactly 50g of sodium 2, 6-dichlorobenzenoneindophenol was dissolved in 150ml of hot water containing 42mg of sodium bicarbonate and diluted with 200ml of water. The solution was then transfered to an amber, glass-stoppered bottle and was then stored in refrigerator at 3^oC. Fresh solution was prepared each week and standardized each day. This solution was used against standard ascorbic acid solution as follows:

A 5ml of aliquot of ascorbic acid solution containing 1mg was diluted with 5ml of 3% metaphosphoric acid. Dye solution was used to titrate, until a pink colour which persists for 15seconds was obtained. This volume of dye solution represents 1mg of ascorbic acid to 1 divided by the number of milliliters used in the titration.

Metaphosphoric acid solution: 60g of metaphosphoric acid beads (HPO_3) in 900ml of water without heating and dilute to 1 liter and stored in a refrigerator. This is a 6% solution. 500ml of

diluted 6% solution was added to 1 liter of water to give a 3% solution.

Procedure: – the equal weights of the sample to 3% metaphosphoric acid. Of this, 200-300g were transferred into an endor and then mixed to obtain homogeneous slurry. The pipette and carriage were used to transfer between 10-30g of the slurry to a 10ml volumetric flask and made to volume with 3% metaphosphoric acid. The mixture was then filtered. Exactly 10ml of the aliquot was transferred into 25ml -50ml of Erlemmeyer flask with the aid of pipette and titrated immediately with the standard solution to a faint pink which persists for 15 seconds. The concentration of ascorbic acid was thereafter calculated.

2.4.10 Determination of Minerals in Samples:
Minerals such as calcium, magnesium, potassium, zinc and iron were determined according to AOAC (1984).

Preparation of the reagent used to determine the minerals are:

Calcium: - About 2.4973g $CaCO_3$ was placed in 1L volumetric flask with 300ml of water. 10ml of HCL was added and after CO_2 has been released and diluted to 1L.

Magnesium: - Exactly 1.000g of Mg was dissolved in 50ml 1M HCL and dilute to 1L with water.

Potassium: - About 1.9067g potassium chloride was dissolved in water and diluted to 1L of H_2O.

Zinc: -Exactly 1.000g of zinc was dissolved in 10ml of HCL and dilute to 1L of water.

Iron: - Exactly 1.000g of Fe wire was dissolved in 20ml of 5ml HCL, diluted to 1L of H_2O.

2.5 Statistical Analysis of Data
The values obtained for each parameter (per sample) were analyzed using means from 3 sample replicates. The mean values were subjected to mean ± S.D at day 3 and 5 and compared with their respective controls.

RESULTS

3.1 Fungal Isolation from samples
The fungi isolated from the infected tomato and 'tatase' samples in Mile 12 market were *Aspergillus niger* and *A. flavus.* The fungi isolated were identified in Plates 1a, 1b, 2a and 2b, which corresponds to their morphological presentation (in culture Plates) and photomicrographs respectively.

3.2 The effect of *A. niger* infection on some vitamins in physically damaged tomato samples

a. Vitamin A (mg/g): The mean value of Vitamin A in the *A. niger* infected samples at day 3 (9.57 ± 0.22^b) and day 5 (6.59 ± 0.17^a) were significantly lower (P = 0.05) than the values obtained in the control sample (12.22 ± 0.37^c). Also, there was a significant difference (P ≤ 0.05) between the Treatment day 3 and Treatment day 5 samples (Figure 1).

b. Vitamin B1 (mg/g): the mean value of Vitamin B1 in the *A.niger* infected samples at day 3 (6.90 ± 0.16^b) and day 5 (4.75 ± 0.12^a) was significantly lower (P = 0.05) than the value obtained in the control sample (8.80 ± 0.26^c). Also, there was a significant difference (P≤0.05) between the Treatment day 3 and Treatment day 5 samples (Figure 1).

c. Vitamin C (mg/g): *A. niger* showed a similar trend (to what obtained in the preceding vitamins) in its deteriorative effect on Vitamin C in the tomato sample. Vitamin C was significantly lower (P = 0.05) in Treatment samples at day 3 (65.77 ± 0.15^b) and day 5 (52.69 ± 0.47^a) compared to the control sample (66.04 ± 0.56^b) (Figure 1).

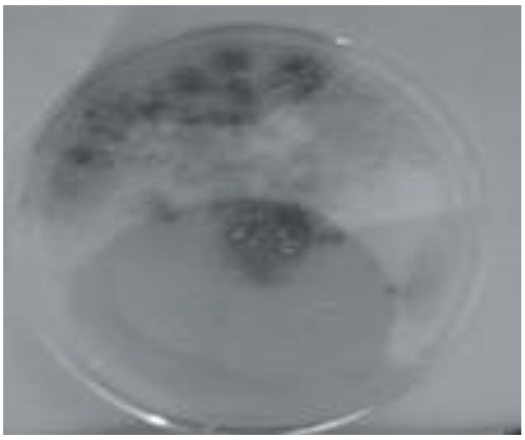

Plate 1a: Culture plate of *A.flavus* isolated from physically damaged *Capsicum annum ('tatase')*

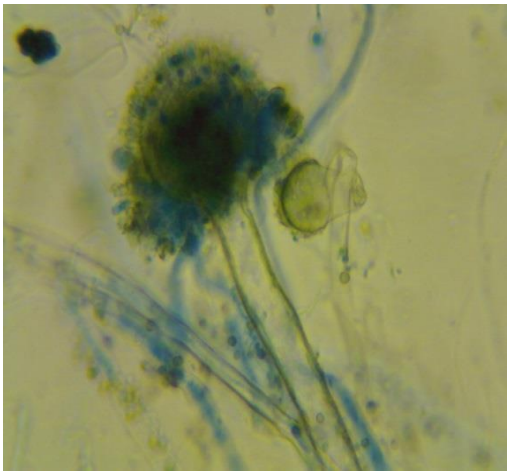

Plate 1b: Photomicrograph of *A.flavus* in Plate 1a

Plate 2a: Culture plate of *A. niger* isolated from Physically damaged tomatoe fruits

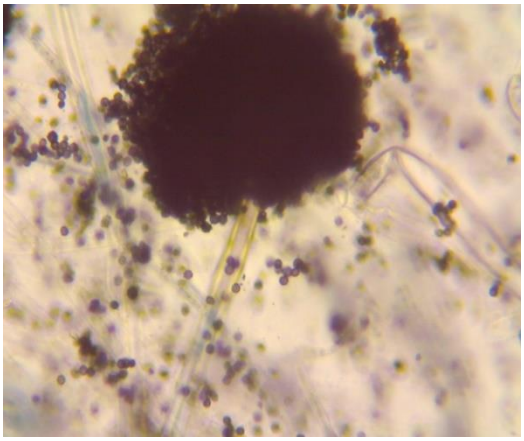

Plate 2b: Photomicrograph of *A.niger* in 2a

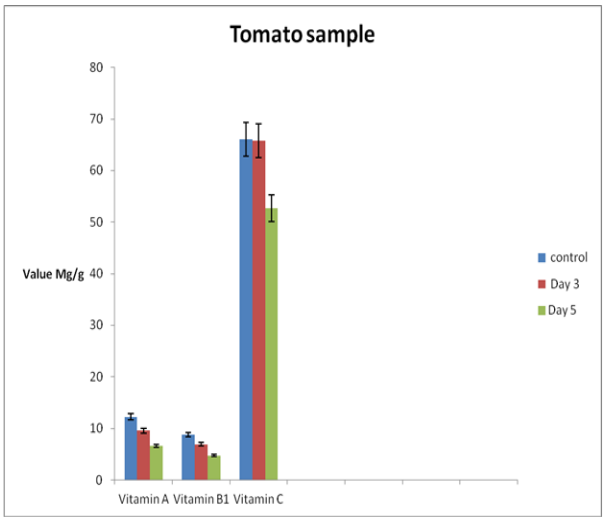

Figure 1: Rate of deterioration of some vitamins by *A.niger* in tomato at days 3 and 5

3.3 The effect of *A. flavus* infection on some vitamins in the physically damaged pepper samples.

a. Vitamin A

A. flavus infection on the 'tatase' sample shows a mean value of 8.32 ± 0.55^{a} at day 3 and 7.79 ± 0.38^{a} at day 5 compared to the control value of 12.99 ± 2.50^{b}. Here, there was no significant difference (P = 0.05) between the mean values of Treatment day 3 and day 5 samples (Figure 2).

b. Vitamin B1: the mean value of Vitamin B1 in *A.flavus* infected samples at day 3 (5.99 ± 0.39^{a}) and day 5 (5.61 ± 0.28^{a}) were significantly lower (P = 0.05) than the values obtained in the control sample (9.35 ± 0.21^{b}). Also, there was no significant difference ($P \leq 0.05$) between the Treatment day 3 and day 5 samples (Figure 2).

c. Vitamin C: the mean value of Vitamin B1 in *A. flavus* infected samples at day 3 (171.42 ± 1.20^{b}) and day 5 (124.02 ± 0.41^{a}) were significantly lower (P = 0.05) than the values obtained in the control sample (173.84 ± 1.21^{b}). However, there was a significant difference (P = 0.05) between the Treatment day 3 and Treatment day5 samples (Figure 2).

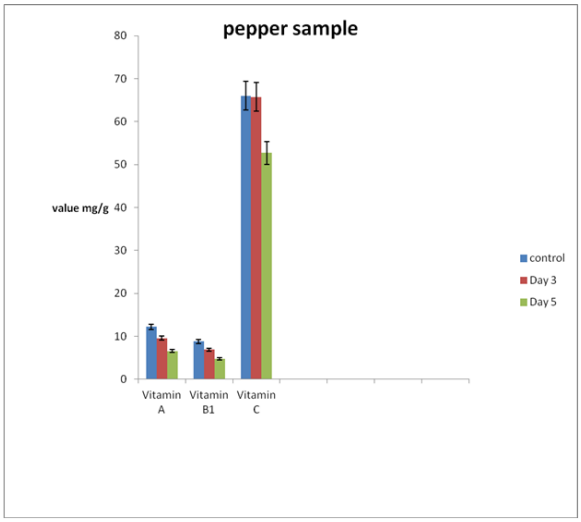

Figure 2: Rate of deterioration in some vitamins by *A.flavus* in pepper at days 3 and 5.

3.4 The effect of *Aspergillus niger* infection on some minerals in physically damaged tomato samples.

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a. Calcium

A.niger showed a similar trend in its deteriorative effect on the mineral composition in the tomato samples as was observed for the vitamin composition in the same sample. The mean value obtained showed calcium in the tomato sample was significantly lower (P = 0.05) in the Treatment samples at day 3 (9.50 ± 0.05^{a}) and 5 (10.30 ± 0.18^{a}) compared with the control sample (12.30 ± 1.06^{b}). here was however no significant difference (P = 0.05), in the mean value of Calcium at day 3 and day 5 (Figure 3a)

b. Magnesium

The results showed that the mean level of Magnesium in the tomato sample was significantly lower (P≤0.05) in the Treatment samples at day 3 (11.10 ± 0.05^{b}) and day 5 (10.47 ± 0.10^{a}) compared to the control (12.05 ± 0.05^{c}). Here, there was a significant difference (P = 0.05) between the Treatment day 3 and day 5 samples (Figure 3a).

c. Potassium

Aspergillus niger caused a similar trend in its deteriorative effect on the potassium in the tomato sample as was observed with calcium. There was no significant difference (P≤0.05) between the mean value for potassium in the Treatment samples at day 3 (172.50 ± 1.00^{a}) and day 5 (173.53 ± 2.52^{a}). The mean value for potassium in the Control Treatment was however significantly higher (P = 0.05) at 180.83 ± 1.53^{b} compared to the values obtained for Treatment day 3 and Treatment day 5 (Figure 3a).

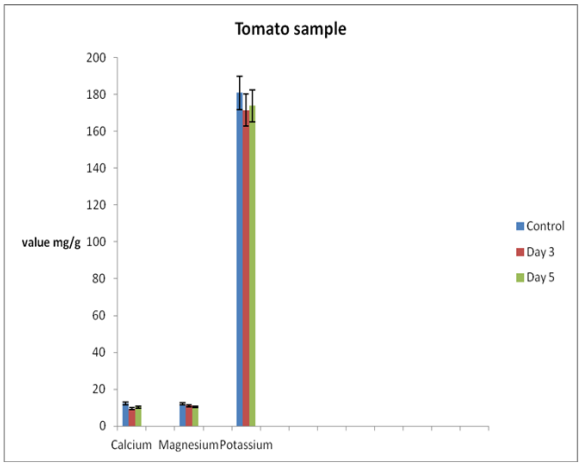

Figure 3a: Rate of deterioration in calcium, magnesium and potassium by *A.niger* in tomato samples at days 3 and 5.

d. Zinc

The results as presented in Figure 3b showed that the mean value of zinc in the tomato sample was significantly lower (P = 0.05) in the Treatment samples at day 3 (0.08 ± 0.001^{a}) and day 5 (0.08 ± 0.003^{a}) compared to control sample (0.09 ± 0.00153^{b}). There was no significant difference (P = 0.05) between the mean values for this element in the Treatment day 3 and day 5 samples.

e. Iron

The mean value of Iron in the tomato sample was significantly lower (P = 0.05) in the Treatment samples at day 3 (0.29 ± 0.01^{a}) and day 5 (0.14 ± 0.01^{b}) compared to control samples (0.48 ± 0.01^{c}). Here, the mean value of Iron obtained for Treatment day 3 sample was significantly higher (P≤0.05) than what was obtained in the Treatment day 5 samples (Figure 3b).

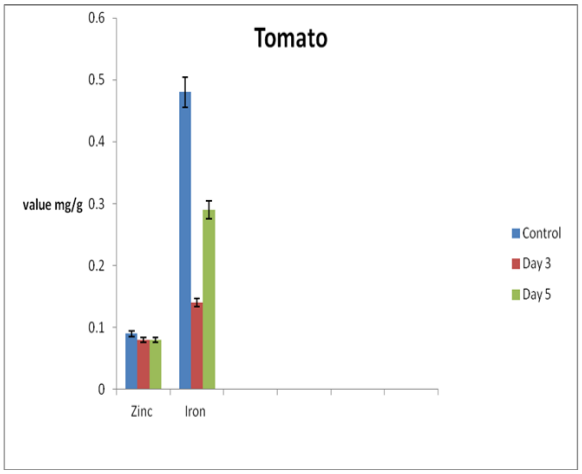

Figure 3b: Rate of deterioration in zinc and iron by *A.niger* in tomato at days 3 and 5

3.5 The effect of *Aspergillus flavus* infection on the mineral composition in pepper fruit.

a. Calcium

The data obtained showed that the value of calcium in the pepper sample was significantly lower (P=.05) in the Treatment samples at day 3 (9.63 ± 0.10^{a}) and day 5 (10.30 ± 0.18^{b}) compared to the control (12.70 ± 0.13^{c}). In addition, calcium was significantly lower (P=.05) in day 3 than in the day 5 samples (Figure 4a)

b. Magnesium

The value of magnesium in the 'tatase' sample was significantly lower (P=.05) in the Treatment samples at day 3(9.63 ± 0.10^{a}) and 5(10.47 ± 0.10^{b}) compared to control sample (13.2 ± 0.3^{c}). However, calcium was significantly lower (P=.05) in the day 3 than in the day 5 samples (Figure 4a)

c. Potassium

Results obtained showed that potassium in the 'tatase' sample was significantly lower (P=.05) in the Treatment samples at day 3(162.5 ± 2.0^{a}) and 5(173.83 ± 2.52^{b}) compared to control sample (180.17 ± 1.5^{c}). In addition, the mean value of potassium found in day 3 sample was significantly lower (P=.05) than in day 5 sample (Figure 4a)

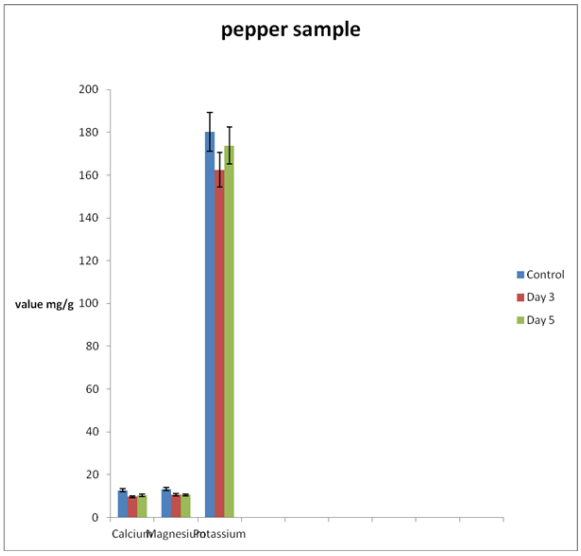

Figure 4a: Deterioration in calcium, magnesium and potassium by *A.flavus* in pepper at days 3 and 5.

d. Zinc

The results obtained showed that *A.flavus* caused the mean value of zinc in the 'tatase' (pepper) sample to be significantly lower (P = .05) at days 3 (0.08 ± 0.001^{a}) and 5 (0.079 ± 0.002^{a}) compared to control (0.09 ± 0.003^{c}). Here, there was no significant difference (P = .05) between the day 3 and 5 samples (Figure 4b)

e. Iron

The value obtained showed Iron in the 'tatase' (pepper) sample was significantly lower (P = .05) at days 3 (0.39 ± 0.02^{b}) and 5 (0.29 ± 0.10^{a}) compared to the control (0.47 ± 0.10^{c}). Also, there was a significant difference (P = .05) between the day 3 and day 5 samples. (Figure 4b).

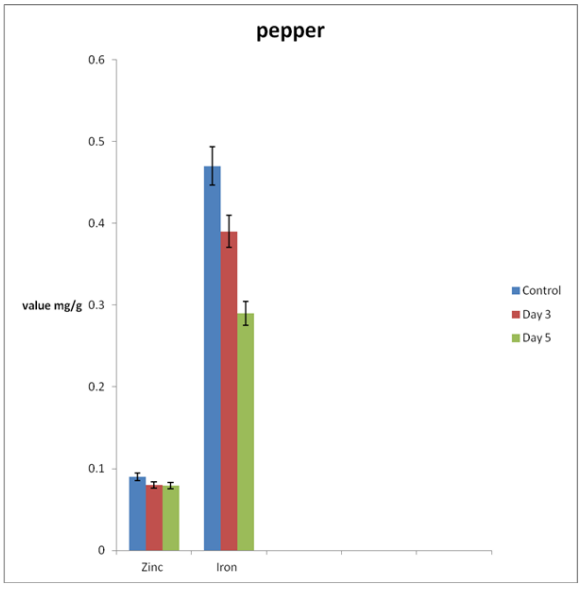

Figure 4b: Rate of deterioration in zinc and iron by *A. flavus* in pepper at days 3 and 5

3.6 The effect of *Aspergillus flavus* on the proximate composition in pepper

a. Protein (mg/g):

Infection of the 'tatase' samples by *A. flavus* showed a similar trend in the deteriorative pattern on the protein content relative to the other types of nutrient in this sample. The mean value obtained showed that there was a significantly lower (P =.05) amount of protein in the Treatment samples at day 3 (8.06 ± 0.25^{b}) and 5(5.91 ± 0.26^{a}) compared to the control sample (9.60 ± 0.26^{c}). In addition, there was a significant difference (P =.05) between the Treatment day 3 and day 5 samples (Figure 5).

b. **Fiber (mg/g):**

A. flavus infection caused a significant increase (P =.05) in the mean level of fiber present in the 'tatase' (pepper) samples at day 3 (9.91 ± 0.40^{b}) and day 5 (12.70 ± 0.57^{c}) when compared to what was found in the control sample (5.06 ± 0.95^{a}). The fiber level found in day 5 samples was equally significantly higher (P=.05) than what was found in the day 3 samples (Figure 5).

c. Carbohydrate (mg/g):

A. flavus caused a significant (P=.05) reduction in the mean value of carbohydrate found in the 'tatase' (pepper) sample at day 3 (31.33 ± 1.86^{b}) and day 5 (23.13 ± 3.11^{a}) when compared to what was found in the control sample (51.00 ± 4.50^{c}). Equally, the mean level of carbohydrate found in the day 5 samples was significantly lower (P =.05) than what was found in the day 3 samples (Figure 5).

d. Energy mg/g:

With respect to the mean energy value, *A. flavus* infection showed a similar trend in its deteriorative effect when compared to the other proximate parameters in the 'tatase' samples. The results showed that the value of energy in the 'tatase' (pepper) sample was significantly lower (P =.05) in the Treatment samples at day 3 (189.18 ± 8.03^{b}) and day 5 (132.38 ± 11.55^{a}) when compared to the control sample (260.94 ± 18.98^{c}). Also, there was a significant difference (P =.05) between the Treatment day 3 and day 5 samples (Figure 5).

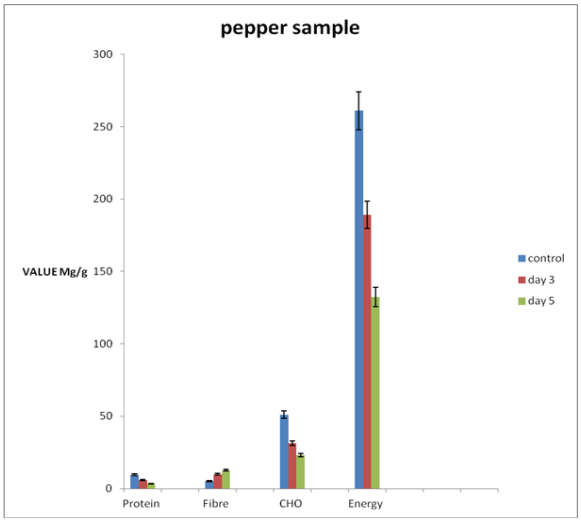

Figure 5: Deterioration in some proximate composition by *A. flavus* in pepper at days 3 and 5

3.7 The effect of *Aspergillus niger* on proximate composition of tomato

a. Protein (mg/g)

A. niger caused a similar trend in its deteriorative effect on the protein level as with the other proximate parameters that were investigated in the tomato samples. The mean value obtained showed that the amount of protein was significantly lower (P = .05) in the Treatment samples at day 3 (7.07 ± 0.11) and day 5 (2.84 ± 0.15^{a}) compared to the control (9.77 ± 0.21^{c}). A further comparison shows that the mean value of protein in the day 3 samples was significantly higher (P = .05) than what was obtained in the day 5 samples (figure 6).

b. Fiber (mg/g)

Infection of the tomato fruits by *A. niger* caused a significant rise (P = .05) in the mean value of fiber at day 3 (8.60 ± 0.23^{b}) and day 5 (10.95 ± 0.45^{c}) compared to the control sample (3.55 ± 1.29^{a}). Also, the fiber content in the day 5 samples was significantly higher (P = .05) than in the day 3 samples (Figure 6).

c. Carbohydrate (mg/g)

A.Niger infection of the tomato fruits caused a significantly lower (P = .05) level of carbohydrate found in the tomato sample at day 3 (46.20 ± 1.11^{b}) and day 5 (29.07 ± 2.25^{a}) when compared to the control sample (62.10 ± 6.60^{c}). Also, the mean value of carbohydrate in the day 5 samples was significantly lower (P = .05) than in the day 3 samples (Figure 6).

d. Energy mg/g

The mean value obtained for energy in the *A.niger* infected tomato sample was significantly lower (P =.05) in the Treatment samples at day 3 (163.19 ± 9.04^{b}) and day 5 (46.20 ± 1.11^{a}) compared to the control samples (306.30 ± 25.84^{c}). In addition, the day 3 samples had a significantly higher (P = .05) mean value of energy than the day 5 samples (Figure 6).

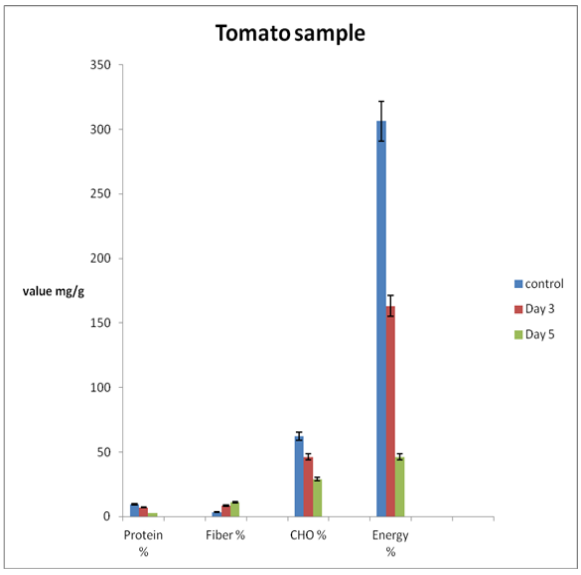

Figure 6: Deterioration in proximate composition by *A.niger* in tomato at days 3 and 5

DISCUSSION

The findings from this research brings to the fore the need for proper packaging and handling of fresh produce and many other food materials that are sold in the Nigerian open markets. The essence of this is to minimize injuries (of mechanical origins) to them. Injuries to plants or plant parts serve as avenues for invasion by pathogenic microorganisms, including mycotoxicogenic fungi. Also, the length of time of storage of these fresh produce appears to be another major factor that supports the growth of fungi (Mohammed *et al*., 2004), and consequently the deterioration in the nutritional worth of the tomato and pepper. It therefore follows that these food materials, under the prevailing storage and handling conditions should not be kept for too long (at most it should be stored for less than a week) before use.

The results from this study agrees with the findings of previous workers where a number of fungal species, including *A.niger* and *A.flavus* were associated with the fruits of *L. esculentum* and *C. annum* either on the field, in the store or in the open markets (Awad, 1990, Adegoke *et al*., 1996 and Kobina and Ebenezer, 2012), where they cause a reduction in the cosmetic and the nutritional value of these vegetables thereby resulting in significant economic losses to the growers of these vegetables (Miedes and Lorences, 2004; Barth *et al*., 2009 and Pandey and Pandey, 2001).

Barth *et al* (2009) reported that microbiological shelf life has become a major reason for sensory quality and shelf life failure for most packaged fresh cut-fruits and vegetables. Microbial spoilage, including off-flavor formation, slimy surface, wetness, soft rot, discoloration, and visual microbial growth/colonies has been used as a main or exclusive objective criterion to determine shelf life of fresh-cut products. In tandem with previous reports (Fatima *et al*., 2009; Pandey and Pandey, 2001 and Kobina and Ebenezer, 2012), the results from this study further reinforces the fact that *A.niger and A.flavus*, are capable of causing a reduction in the nutritional quality of pepper and tomato fruits.

Rice *et al*., (1991) and Amusa *et al*. (2003) reported an increase in some mineral contents in infected fruits, an observation which was similar to some of the results obtained in this study, particularly, where infection by *A. niger* and *A. flavus* resulted to an increase in the mean value of calcium and potassium for the day 5 samples.

In addition, the reduction in the protein and carbohydrate content of these vegetables as reported in this work agrees with the findings of Rice *et al*. (1991) and Amusa *et al* (2003) where some fungal species were reported to have caused some deterioration specifically in the protein and carbohydrate content in some fruits and vegetables. The import of this finding is with respect to the formal/Western food industry, where this finding might have a remarkable effect on the value of the vegetables from this part of the world.

With respect to the depletion of vitamin C due to the activities of the fungal species reported in this study, previous reports have also shown a depletion in the content this important vitamin (in some other fruits and vegetables) due to the activities of *A. niger* (Tendon, 1998) and *Botryodiplodia theobromae* (Arya, 1993).

The results from this study showed that *A. niger* and *A. flavus* caused a deterioration in all the vitamins evaluated in both vegetable samples. It should be noted however that vitamins A, B1, and C are essential nutrients that are required for normal body functioning (Khana *et al.,* 2003). Vitamin A is important for healthy bones, teeth, mucus membranes and skin. In addition, its aids vision especially in the dark. Protein helps in the building up of new cells in the body and enhances growth. Fat in the diet serves as a source of energy in the body. Calcium aids the formation of bones, while iron in the diet serves as a source of blood formation (South Pacific Foods, 1995).

Lack of vitamin C in diet can lead to deficiencies like weakness, lassitude, swollen gums and sometimes bleeding gums, nose bleeding, swollen or painful joints, paleness and sometimes the inability to synthesize vitamin E (McDowell, 2000).

It is important to stress that species of *Aspergillus* and other fungi such as *Penicillium* and *Alternaria* have been indicted as the major contributors to fruit and vegetable decay as well as the production of mycotoxins such as aflatoxins, patulin, ochratoxin A (OTA) and *Alternaria* toxins (Barkai-Golan and Paster, 2008). *A. flavus* in particular have been associated with the production of aflatoxin while *A.niger* and *A.flavus* have both been indicted with the production of OTA, both of which are known carcinogens (Barkai-Golan, 2008). Other health concerns associated with mycotoxin contamination of food include but not limited to mycotoxicoses, liver damage, suppression of the immune system and death (Zain, 2011).

Apart from the risk of food poisoning that can be triggered by the deposition of toxins by these fungi (that are associated with these physically damaged produce), the fact that they are also inferior in terms of their nutritional content when compared to the healthy undamaged produce should naturally make the physically undamaged tomato and "tatase" (pepper) a preferred choice.

Conclusion

The findings from this work have burst the myth that physically damaged (i.e. "esa") tomato and "tatase" (pepper) are not inferior to the fresh undamaged tomatoes and pepper in terms of nutritional worth. Finally, this research has also brought to the fore the need for a stricter regulation and monitoring of the myriad road side restaurants that dot the Nigerian landscape. This is because to a large extent, these group of service providers are more culpable in the use of these physically damaged food condiments (because they are by far cheaper than the visually healthy ones) that can potentially jeopardize the health of the Nigerian people.

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